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Disciplines

Comparative and Laboratory Animal Medicine | Medicine and Health Sciences | Ophthalmology | Veterinary Medicine

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Cloning and Characterization of the cDNA Encoding the α -Subunit of cGMP-Phosphodiesterase in Canine Retinal Rod Photoreceptor Cells

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Rod photoreceptor cyclic GMP-phosphodiesterase (cGMP-PDE, EC 3.1.4.17) is a key enzyme in the phototransduction cascade of the vertebrate retina. The enzyme is composed of α - and beta-catalytic subunits, and two identical inhibitory gamma-subunits. Once the phototransduction cascade is initiated by the absorption of light by rhodopsin, activated cGMP-PDE rapidly hydrolyses cGMP, depletion of which shuts cGMP-gated cation channels in the plasma membrane. The consequent hyperpolarization of the photoreceptor outer segment represents a large signal amplification and generates the visual neural impulse. Aberrant function of cGMP-PDE is causally associated with retinal degenerative diseases in man and animals. Mutations in the genes for the α - and beta-subunits of cGMP-PDE (*PDEA* and *PDE6B*, respectively) cause retinitis pigmentosa (OMIM entry) in some human families [6,8,9]. Defects in *PDE6B* also cause retinal degeneration in the *rd* mouse [3,11] and the *rd1* dog [5,13,14]. Rod-cone dysplasia 2 (*rcd2*), which affects the collie dog, also represents a defect of retinal cyclic GMP metabolism since retinal cGMP levels are significantly elevated and cGMP-PDE activity is deficient [16]; however, *rcd2* is not caused by a defect in *PDE6B*[1]. Thus it is likely that the *rcd2* locus codes for either another cGMP-PDE structural subunit [*PDEA*, or *PDEG* (the gene for the gamma-subunit of cGMP-PDE)], or for one of the other proteins in the phototransduction cascade that activates cGMP-PDE. Since the canine chromosomal locations of the *rcd2* locus and the genes involved in phototransduction are not known, none of these candidate genes can be ruled out based on their map location relative to the *rcd2* locus.

In order to identify the mutation responsible for the *rcd2* disorder, we have begun to examine the different candidate genes that code for the phototransduction cascade proteins in the dog. The cDNAs for the α -subunit of cGMP-PDE (*PDE α*) have been cloned and characterized from man [12], mouse [2], and cow [10,12]. However, no information is available in the literature on the canine *PDE α* cDNA.

In this study we characterize the canine *PDE α* cDNA from normal dog, present evidence for usage of alternate polyadenylation sites to generate the two different transcripts described in multiple species [2,7,12], and compare the deduced amino acid sequences for conservation through evolution.

RESULTS

Initially two different segments of canine *PDE α* cDNA were obtained by reverse transcription (RT) and polymerase chain reaction (PCR) using total retinal RNA and consensus primer pairs (*PDEA*-1/*PDEA*-2 and *PDEA*-3/*PDEA*-4; Table 1) based on the coding regions of known *PDE α* cDNA sequences from other species. These RT-PCR amplified fragments were cloned (*1-PDEA* and *2-PDEA*) in pCRII vector (Invitrogen; San Diego, CA) and sequenced to confirm authenticity of the retina-specific *PDE* cDNA sequence. From the confirmed canine

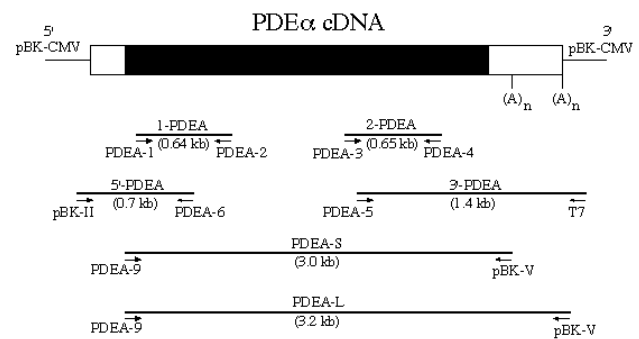


Figure 1. Strategy for cloning the canine *PDE α* cDNA. The cDNA containing the coding sequence (shaded box), the untranslated regions at the 5'- and 3'-end (open boxes) and two sites where poly (A) are added, is shown as an insert in the vector pBK-CMV (Stratagene, La Jolla, CA) used for the construction of the library. Clones containing different overlapping regions of the cDNA (*1-PDEA*, *2-PDEA*, *5'-PDEA*, *3'-PDEA*, *PDEA-S* and *PDEA-L*), the sizes of the cDNA fragments in the clones, and the primers (see Table 1 for sequence) used for generating those fragments are identified. The clones *1-PDEA* and *2-PDEA* were obtained by reverse transcription and polymerase chain reaction (RT-PCR) from canine retinal total RNA using the RT-PCR kit (Perkin-Elmer; Foster City, CA) as recommended by the manufacturer. Amplification of cDNA to obtain *1-PDEA* and *2-PDEA* was done for 30 cycles at 94 °C (1 min), 54 °C (2 min), 72 °C (2 min). Other clones (*5'-PDEA*, *3'-PDEA*, *PDEA-S* and *PDEA-L*) were obtained by screening a canine retinal cDNA library using a PCR based method. The PCR for screening the canine retinal cDNA library was done in 50 μ l containing 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 0.2 mM dNTPs, 2.0 mM MgCl₂, 10% DMSO, and 1.25 units Taq polymerase (Life Technologies; Grand Island, NY). The conditions for PCR amplification of each fragment were as follows: *5'-PDEA* and *3'-PDEA* were obtained by 30 cycles at 94 °C (1 min), 60 °C (1.5 min), 72 °C (2 min); *PDEA-S* and *PDEA-L* were obtained by 30 cycles at 94 °C (1 min), 58 °C (2 min), 72 °C (3 min). All reactions were concluded with a single step extension reaction at 72 °C for 10 min.

PDE α cDNA sequence, new canine-specific primers were designed to amplify both the 5'- and 3'-ends of canine PDE α cDNA from a canine retinal cDNA library by PCR (Figure 1).

To amplify the 5'-end of the cDNA, forward vector-specific (pBK-II) and PDEA-specific reverse (PDEA-6) primers were used. From the PCR products, the largest fragment showing evidence for PDEA-specificity, based on PCR using internal primers, was cloned (5'-PDEA). To amplify the 3'-end of the cDNA, gene specific forward (PDEA-5) and vector specific reverse (T7) primers were used, and the amplified DNA fragment was cloned (3'-PDEA). The clone 3'-PDEA, however, lacked the poly (A) tail. We therefore designed a canine-specific primer (PDEA-9) from the 5'-end of the cDNA and used it in combination with the vector-specific reverse primer (pBK-V) for amplification of the entire open reading frame (ORF) and 3'-untranslated region (UTR) of PDE α cDNA from the cDNA library. The PCR resulted in amplification of two DNA fragments (3.0 and 3.2 kb), both of which hybridized to canine PDE α cDNA in Southern blots (data not shown). These two putative PDE α cDNA fragments (PDEA-L and PDEA-S) were cloned as described above.

Sequence of the PDE α cDNA was obtained from clones 1-PDEA, 2-PDEA, 5'-PDEA, 3'-PDEA, and PDEA-S, which contain overlapping fragments, from both directions. The identity of the larger clone (PDEA-L) as PDE α cDNA was confirmed by (a) partial sequencing (800 bp) of the 3'-end and two other upstream regions of the insert; (b) amplification of multiple overlapping fragments identical in size to those obtained from PDEA-S by PCR using the same set of primers; and (c) identical and predicted restriction enzyme digestion pattern of PCR amplified DNA fragments from both the clones (Figure 2). Sequences of all the primers used and their location in the canine PDE α cDNA or vector DNA are listed in

TABLE 1. SEQUENCE AND LOCATION OF PRIMERS USED FOR PCR

| Primer sequence(5'to3') | Source of Primer | Name of primer | Location |
|----------------------------|--------------------|----------------|-----------|
| gCTTTGCCAACAGTACTCAACC | hPDE α cDNA | PDEA1 | 192-215 |
| AgTTTGTGcAGGTAACCTCAGGTG | hPDE α cDNA | PDEA2 | 815-836 |
| GGCCCTGGTGCgGTTC | hPDE α cDNA | PDEA3 | 1759-1774 |
| ATGGGATTTCTGTGcAGCAC | hPDE α cDNA | PDEA4 | 2393-2412 |
| TTCAACGTGGGGcAGACCAT | cPDE α cDNA | PDEA5 | 1832-1851 |
| CACTACGTCTTCCcATTTCATTATGG | cPDE α cDNA | PDEA6 | 681-706 |
| GTCATaAGAAGCTGTGCTTCCTCC | cPDE α cDNA | PDEA7 | 380-404 |
| GTCATGGGTGAGGTGcAGCAGAG | cPDE α cDNA | PDEA9 | 137-160 |
| cCAACGTTTTGCCGAACCTCCAAG | cPDE α cDNA | PDEA11 | 2032-2054 |
| TCCACCCCTATTCTGGTCCCA | cPDE α cDNA | PDEA16 | 1036-1055 |
| ATGGTCTGCCCCACGTTGAAGCC | cPDE α cDNA | PDEA17 | 1829-1851 |
| TTGCTTGGCTGTCTGTGCTT | cPDE α cDNA | PDEA18 | 2624-2644 |
| GCAGGTGCACACTAGTGGATCC | pBKCMV | pBKII | 1092-1113 |
| CCGCTCTAGAAGTACTCTCGAGTT | pBKCMV | pBKV | 1052-1067 |
| CGACTCACTATAGGGCAATT | pBKCMV | T7 | 980-1000 |

Primers (PDEA-1 to PDEA-4) used for RT-PCR correspond to the human (h) PDE α cDNA sequence, selected from the consensus region in different species. All other PDEA primers correspond to canine (c) PDE α cDNA sequence. Location of all the PDEA primers are shown with respect to cPDE α cDNA sequence. Nucleotides (shown in lower case) in some primers have mismatches with the canine sequence because those primers were selected either from the human sequence, or from preliminary canine sequence. Phagemid vector (pBK-CMV) specific primers were selected either from the multiple cloning site or from the flanking region. The bold-italicized region of primer pBK-V represents the linker used to make the canine cDNA library.

Table 1.

The two full length canine rod PDE α cDNAs are 2988 and 3233 nucleotides long, including the poly (A) tail (Figure 3). We have previously reported the presence of two distinct PDE α mRNAs, 3.3 and 3.0 kb, expressed in equivalent amounts in canine retina by northern blot [7]. Thus the sizes of the canine PDE α cDNAs described here (GenBank accession number U52868) are in complete agreement with our observation from northern analysis [7]. We have not formally determined the transcription start site; however, the clone containing the most upstream 5'-noncoding region contains 139 nucleotides in the 5'-UTR followed by the ATG codon for initiation of translation. The stop codon (TAA) corresponds to positions 2723 through 2725 of the cDNA sequence. The 3'-UTRs of the 3.0 kb and 3.3 kb transcripts, represented by clones PDEA-S and PDEA-L, are 263 and 508 nucleotides respectively. A non-consensus putative polyadenylation signal (ATTAAA) is present in the corresponding locations of the smaller (PDEA-S) and larger (PDEA-L) clones. In PDEA-S this first polyadenylation signal (Figure 3, nucleotides 2948 through 2953) is 15 nucleotides upstream of the poly (A) tail.

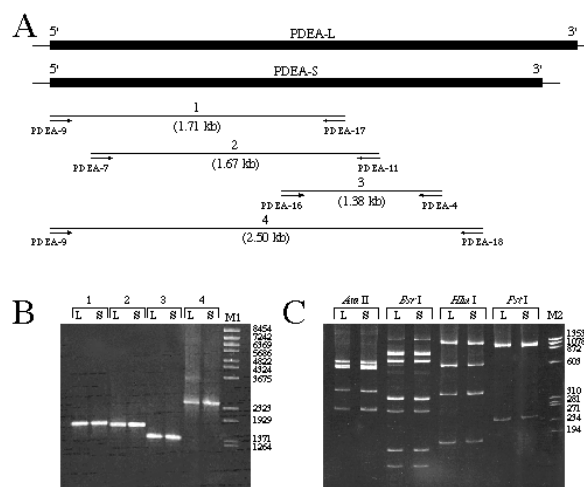


Figure 2. Identity of PDEA-L clone as PDEa cDNA sequence. (A) Relative size of the inserts in the PDEA-L and PDEA-S clones, the DNA fragments amplified by PCR, primers (see Table 1 for sequence) used, and the predicted sizes of the amplified fragments are shown. PCR condition for amplified fragments are as follows: fragments 1 and 2 were obtained by 30 cycles at 94 °C (1 min), 64 °C (1 min), 72 °C (2 min); fragment 3 was obtained by 30 cycles at 94 °C (1 min), 54 °C (1 min), 72 °C (1.5 min); and fragment 4 was obtained by 35 cycles at 94 °C (1 min), 58 °C (2 min), 72 °C (3.5 min). All reactions were concluded with a single step extension reaction at 72 °C for 5 min.

(B) Agarose gel (0.8%) electrophoresis of PCR products from PDEA-S (S) and PDEA-L (L) clones. Numbers 1 through 4 identify DNA fragments amplified from regions of the clones represented in panel A. Lane M1 represents BstE II-digested lambda DNA markers (C) Polyacrylamide gel (6%) electrophoresis of restriction enzyme digestion of PCR product (4 in panel A) from PDEA-S (S) and PDEA-L (L) clones. Lane M2 represents Hae III-digested phi X 174 markers. The length of the DNA fragments (bp) in both markers (M1 and M2) are shown next to the marker lanes. The PCR and the restriction enzyme digestion results indicate that both the clones are identical in the regions examined.

In the larger clone (PDEA-L) there is a second polyadenylation signal (Figure 3, nucleotides 3187 through 3192) 23 nucleotides upstream of the poly (A) tail. The ORF of the canine sequence predicts a protein of 99.7 kDa containing 861 amino acids.

DISCUSSION

Comparison of the ORF nucleotides of the canine PDE α cDNA with that of other species shows that it shares similar nucleotide identity with the bovine (91.0%) and human (90.4%) sequences, and slightly lower identity with the mouse (86.5%) sequence. At the amino acid level, however, the similarity between the canine sequence and that of other species is higher

(94%) than at the nucleotide level. Two transcripts have been reported to be present for human (4.9 and 5.3 kb), bovine (4.0 and 4.6 kb) and canine (3.0 and 3.3 kb) PDE α [2,7,12]. The reported human and bovine clones, however, contain only 2.9-3.2 kb of the PDE α cDNA sequence [10,12]. The size of the transcript in mouse has not been reported. Since only a single ORF with high homology between different species has been identified for PDE α cDNAs, it is reasonable that the inter- and intra-species difference in the sizes of the transcripts could be due to either different transcription start sites or use of alternative poly (A) addition sites. The sequence presented here clearly demonstrates that two differently sized canine PDE α transcripts are generated by use of different polyadenylation

| | | | |
|---|------|--|------|
| CTTCGAGGTCGACACTAGTGGATCCAAAGAATTTCGGCACGAGAAAACCTCTGACTCT | 60 | GAC AAT GAA GAG ATC CAG AAA ATC CTG AAA ACC AGA GAG GTG TAT | 1564 |
| GTCTTGCCCCAGCTATAGACATTCCTCCCTGGGCGAGCCAGCTTTAGGCTCTCTGGGAAGT | 120 | Asp Asn Glu Glu Ile Gln Lys Ile Leu Lys Thr Arg Glu Val Tyr | 475 |
| AGCCAGCGGGATCCAGTC ATG GGT GAG GTG ACA GCA GAG CAG GTG GAG | 169 | GGG AAG GAG CCG TGG GAG TGC GAG GAA GAG GAA CTC GCT GAG ATC | 1609 |
| | | Gly Lys Glu Pro Try Glu Cys Glu Glu Glu Leu Ala Glu Ile | 490 |
| AAG TTC CTG GAC TCG AAT ATT ATC TTT GCC AAA CAG TAC TAC AAC | 214 | CTG CAA GGA GAG CTG CCA GAT GCA GAG AAA TAT GAA ATC AAT AAA | 1654 |
| Lys Phe Leu Asp Ser Asn Ile Ile Phe Ala Lys Gln Tyr Tyr Asn | 25 | Leu Gln Gly Glu Leu Pro Asp Ala Glu Lys Tyr Ile Asn Lys | 505 |
| CTC CGC TAC CGG GCC AAG GTC ATC TCA GAC ATG CTG GGG GCC AAG | 259 | TTC CAC TTC AGC GAC TTG CCC CTG ACC GAA CTG GAG CTG GTG AAA | 1699 |
| Leu Arg Tyr Arg Ala Lys Val Ile Ser Asp Met Leu Gly Ala Lys | 40 | Phe His Phe Ser Asp Leu Pro Leu Thr Glu Leu Glu Leu Lys | 520 |
| GAG GCA GCG GTG GAC TTC AGC AAC TAC CAC TCG CTG AGC AGT GTG | 304 | TGT GGG ATA CAG ATG TAC TAT GAG CTC AAA GTG GTG GAT AAA TTT | 1744 |
| Glu Ala Ala Val Asp Phe Ser Asn Tyr His Ser Leu Ser Ser Val | 55 | Cys Gly Ile Gln Met Tyr Tyr Glu Leu Lys Val Val Asp Lys Phe | 535 |
| GAG GAG AGT GAA ATC ATC TTT GAG CTC CTG CGA GAC TTC CAG GAG | 349 | CAC ATT CCT CAG GAG GCC CTG GTG CGC TTC ATG TAC TCG CTG AGC | 1789 |
| Glu Glu Ser Glu Ile Ile Phe Asp Leu Leu Arg Asp Phe Gln Glu | 70 | His Ile Pro Gln Glu Ala Leu Val Arg Phe Met Tyr Ser Leu Ser | 550 |
| AAT TTG CAG GCC GAG AGA TGC ATC TTC AAT GTC ATG AAG AAG CTG | 394 | AAG GGC TAC CGC AGG ATC ACC TAC CAC AAC TGG CGG CAC GGC TTC | 1834 |
| Asn Leu Gln Ala Glu Arg Cys Ile Phe Asn Val Met Lys Lys Leu | 85 | Lys Gly Tyr Pro Arg Ile Thr Tyr His Asn Try Arg His Gly Phe | 565 |
| TGC TTC CTC TTT CAG GCA GAT CGC ATG AGC CTG TTC ATG TAC AGG | 439 | AAC GTG GGG CAG ACC ATG TTC TCC TTG CTG GTG ACC GGA AAG CTG | 1879 |
| Cys Phe Leu Leu Gln Ala Asp Arg Met Ser Leu Phe Met Tyr Arg | 100 | Asn Val Gly Gln Thr Met Phe Ser Leu Leu Thr Thr Gly Lys Leu | 580 |
| GTC CGA AAT GCC ATC GCA GAG CTA GCC ACC CGG CTC TTC AAT GTC | 484 | AAG CGA TAC TTC ACA GAC CTA GAG GCC TTG GCC ATG GTC ACC GTC | 1924 |
| Val Arg Asn Gly Ile Ala Glu Leu Ala Thr Arg Leu Phe Asn Val | 115 | Lys Arg Tyr Phe Thr Asp Leu Glu Ala Leu Ala Met Val Thr Ala | 595 |
| CAC AAG GAT GCT GTC CTT GAG GAA TGC CTG GTG GCG CCC GAC TCA | 529 | GCC TTC TGC CAT GAC ATT GAC CAC AGA GGC ACC AAC AAT CTC TAC | 1969 |
| His Lys Asp Ala Val Leu Glu Glu Cys Leu Val Ala Pro Asp Ser | 130 | Ala Phe Cys His Asp Ile Asp His Arg Gly Thr Asn Asn Leu Tyr | 610 |
| GAG ATT GTG TTC CCC CTG GAC ATG GGT GTG GGT CAC GTT GCC | 574 | CAG ATT AAG TCC CAG AAC CCA CTG GCC AAG CTC CAT GGT TCC TCC | 2014 |
| Glu Ile Val Phe Pro Leu Asp Met Gly Val Val Gly His Val Ala | 145 | Gln Met Lys Ser Gln Asn Pro Leu Ala Lys Leu His Gly Ser Ser | 625 |
| CAC TCT AAA AAG ATC GCC AAG GTC AAT ACA AAG GAG GAG | 619 | ATC TTG TAA AGA CAC CAC TTG GAG TTC GAG AAA ACG TGT CTG CGA | 2059 |
| His Ser Lys Lys Ile Ala Asn Val Val Asn Thr Glu Glu Asp Glu | 160 | Ile Leu Glu Arg His His Leu Glu Phe Gly Lys Thr Leu Leu Arg | 640 |
| CAT TTC TGT GAC TTT GTG GAC ACC CTC ACT GAG TAC CAG ACC AAG | 664 | GAT GAG AGC CTG AAT ATC TTT CAA AAC CTC AAT CGC AGG CAG CAC | 2104 |
| His Phe Cys Asp Phe Val Asp Thr Ser Thr Glu Tyr Gln Thr Lys | 175 | Asp Gly Ser Leu Asn Ile Phe Gln Asn Leu Asn Arg Arg Gln His | 655 |
| AAC ATC CTG GCT TCC CCC ATA ATG AAT GGG AAG GAC GTA GTG GCA | 709 | GAG CAC GCC ATC CAC ATG ATG GAC ATA GCA ATT ATT GCC ACA GAC | 2149 |
| Asn Ile Leu Ala Ser Pro Ile Met Asn Gly Lys Asp Val Val Ala | 190 | Glu His Ala Ile His Met Met Asp Ile Ala Ile Ala Met Val Thr | 670 |
| GTA ATC ATG GCT GTG AAT AAA GTG GAC GAG CCC CAC TTC ACC AAG | 754 | CTC GCC CTG TAT TTC AAG AAG AGG ACA ATG TTC CAA AAG ATC GTG | 2194 |
| Val Ile Met Ala Val Asn Lys Asp Asp Glu Pro His Phe Thr Lys | 205 | Leu Ala Leu Tyr Phe Lys Lys Arg Thr Met Phe Gln Lys Ile Val | 685 |
| AGA GAT GAA GAG ATT CTT CTC AAG TAC CTC AAT TTT GCA AAC TCA | 799 | GAT CAG TCT AAA ACA TAT GAA ACT CAG CAG Phe TGG ACA CAG TAC | 2239 |
| Arg Asp Glu Glu Ile Leu Leu Lys Tyr Leu Asn Phe Ala Asn Leu | 220 | Asp Gln Ser Lys Thr Tyr Glu Thr Gln Gln Glu Lys Thr Lys Tyr | 700 |
| ATC ATG AAG GTA TAC CAC CTG AGT TAC CAC AAT TGC GAG ACT | 844 | ATG ATG CTG GAG CAG ACA CGG AAG GAA ATT GTT ATG GOC ATG | 2284 |
| Ile Met Lys Val Tyr His Leu Ser Tyr Leu His Asn Cys Glu Thr | 235 | Met Met Leu Glu Gln Thr Arg Lys Glu Ile Val Met Ala Met Met | 715 |
| CGG CGT GGC CAG ATA CTG CTG TGG TCT GGG AGC AAA GTC TTT GAA | 889 | ATG ACC GCC TGT GAT CTC TCA GCC ATC ACC AAG CCC TGG GAG GTG | 2329 |
| Arg Arg Gly Gln Ile Leu Leu Tyr Ser Gly Ser Lys Val Phe Glu | 250 | Met Thr Ala Cys Asp Leu Ser Ala Ile Thr Lys Pro Try Glu Val | 730 |
| GAG CTT ACG GAC ATC GAG AGG CAG TTC CAC AAG GCC CTG TAC ACA | 934 | CAG AGT AAG GTA GCT CTA CTG GTT GCT GCC GAA TTC TGG GAA CAA | 2374 |
| Glu Leu Thr Asp Ser Ile Glu Arg Gln Phe His Lys Ala Leu Tyr Thr | 265 | Gln Ser Lys Val Ala Leu Leu Val Ala Ala Glu Phe Tyr Glu Lys | 745 |
| GTC CGG GCC TTC CTC AAC TGT GAC AGA TAT TCT GTG GGA CTC TTA | 979 | GGT GAC CTG GAG CGC ACA GTG CTG CAG CAG AAT CCC ATT CCC ATG | 2419 |
| Val Arg Ala Phe Leu Asn Cys Asp Arg Tyr Ser Val Gly Leu Leu | 280 | Gly Asp Leu Glu Arg Thr Val Leu Gln Gln Asn Pro Ile Pro Met | 760 |
| GAC ATG ACC AAG CAG AAG GAA TTT TTT GAT GTG TGG CCA GTC CTG | 1024 | ATG GAC AGG AAC AAG GCA GAT GAA CTC CCC AAG CTT CAA GTC GGC | 2464 |
| Asp Met Thr Lys Gln Lys Glu Phe Phe Asp Val Try Pro Val Leu | 295 | Met Asp Arg Asn Lys Ala Asp Glu Leu Pro Lys Leu Gln Val Gly | 775 |
| ATG GGG GAG GCT CCA CCC TAT TCT GGT CCC AGG ACT CCG GAT GGA | 1069 | TTC ATT GAC TTT GTT TGC ACC TTT GTC TAC AAG GAA TTC TCC GGT | 2509 |
| Met Gly Glu Ala Pro Pro Tyr Ser Gly Pro Arg Thr Pro Asp Gly | 310 | Phe Ile Asp Phe Val Cys Thr Phe Val Tyr Lys Glu Phe Ser Arg | 790 |
| AGG GAA ATC AAC TTT TAC AAG GTC ATT GAC TAT ATC CTA CAC GGC | 1114 | TTC CAC GAG GAG ATC ACT CCC ATG CTG GAT GGG ATC ACC AAC AAC | 2554 |
| Arg Glu Ile Asn Phe Tyr Lys Val Ile Asp Tyr Ile Leu His Gly | 325 | Phe His Glu Glu Ile Thr Pro Met Leu Asp Gly Ile Thr Asn Asn | 805 |
| AAA GAA GAC ATC AAA GTA ATC CCG AAT CCA CCT CCT GAT CAT TGG | 1159 | CGC AAG GAG TGG AAG GCG CTC GCC GAT GAG TAC CAG ACC AAG | 2599 |
| Lys Glu Asp Ile Lys Val Ile Pro Asn Pro Pro Pro Asp His Try | 340 | Arg Lys Glu Try Lys Ala Leu Ala Asp Glu Tyr Asp Thr Lys Met | 820 |
| GCT TTA GTA AGT GGT CTG CCC ACT TAT GTT GCC CAG AAT GGC CTG | 1204 | AAG GCC CTG GAG GAG AAG CAG AAG CAG CAG ACA GCC AAG CAA | 2644 |
| Ala Leu Val Ser Gly Leu Pro Thr Tyr Val Ala Gln Asn Gly Leu | 355 | Lys Ala Leu Glu Glu Glu Lys Gln Lys Gln Thr Ala Lys Gln | 835 |
| ATT TGC AAC ATC ATG AAT GCA CCT GCA GAG GAC TTT TTT GAA TTC | 1249 | GGG GCG GCA GGA GAT CAG CCG GGG GGC AAC CCC ACG GCG GGG | 2689 |
| Ile Cys Asn Ile Met Asn Ala Pro Ala Glu Asp Phe Phe Ala Phe | 370 | Gly Ala Ala Gly Asp Gln Pro Gly Gly Asn Pro Ser Pro Ala Gly | 850 |
| CAG AAA GAG CCT CTG GAT GAG TCT GGA TGG ATG ATT AAG AAT GTC | 1294 | GGC GCA CCT GCA TCC AAG TCC TGC TGC ATC CAG TAA CGGTGCTGGCA | 2737 |
| Gln Lys Glu Pro Leu Asp Glu Ser Gly Try Met Ile Lys Asn Val | 385 | Gly Ala Pro Ala Ser Lys Ser Cys Cys Ile Gln *** | 2761 |
| CTT TCT TTG CCA ATT GTG AAC AAG AAG GAG GAA ATT GTT GGG GTG | 1339 | TCAGCTGACCCAGTGGTACCACCCCTTCTCTGGAAAGAGACCACCCAGCCAGCAGAAAAAC | 2897 |
| Leu Ser Leu Pro Ile Val Asn Lys Lys Glu Ile Val Gly Val | 400 | CAAAACCCCTGCTTGTGAAGTAAAAATAGTAATCGGATTTTGAAGCTGGGAGAGAAATTTAGC | 2857 |
| GCC ACG TTT TAC AAT CGC AAA GAT GGA AAA CCC TTT GAT GAA ATC | 1384 | TTACTTTCTACTAGTGGTTTTTGAACATTTTTTCAGTTTTTGAATCTTTTACTGAGCTA | 2917 |
| Ala Thr Phe Tyr Asn Arg Lys Asp Gly Lys Pro Phe Asp Gly Met | 415 | AAACCAACATCTAGCTTTAATAGACATCAATTAACATTTAATTAAGGCCAAGTTCAATC | 2977 |
| GAT GAG ACC CTC ATG GAG TCT TTG GCT CAA TTC CTG GGC TGG TCC | 1429 | TGCTTGCTTAGAATCATTTTTTCACTCTTATACTTCCATTTTTATGATTTCTTATAACAATTC | 3037 |
| Asp Glu Thr Leu Met Glu Ser Leu Ala Gln Phe Leu Gly Tyr Ser | 430 | TCTAGCATCTCAAAAAGTAGAGAATTTGTTCAAGTAAATCCCTCTCCATCACCAGATTC | 3097 |
| GTC TTA AAT CCT GAT ACT TAC GAG TCA ATG AAC AGA TCT GAA AAC | 1474 | CCAAATCAGGGTTTTGCCCCATTTGCTTCATCCATCTTTTACATTTCTTTTCGCATTTT | 3157 |
| Val Leu Asn Pro Asp Thr Tyr Glu Ser Met Asn Arg Leu Glu Asn | 445 | TTTCTTAGCTGAAGTGAAGTATTTTCTTTTAAATATGATGGTCTTTTTTCTTTTTTAA | 3217 |
| AGG AAG GAT ATT TTC CAG GAC ATG GTA AAA TAC CAC GTG AAG TGT | 1519 | AAAAAAAAAAAAAAAAAAAA | 3233 |
| Arg Lys Asp Ile Phe Gln Asp Met Val Lys Tyr His Val Lys Cys | 460 | | |

Figure 3. The nucleotide sequence of PDE α cDNA from canine retina (GenBank accession number U52868), and the deduced amino acid sequence. The position of the nucleotides and the deduced amino acids are indicated on the right. The two extra amino acids that are inserted in comparison to human and mouse sequences are shown in **bold italics** [**Ala** (849), **Gly** (850)]. The stop codon (TAA) is marked with asterisks (***), and the sequence motif (ATTAAA) present at the two polyadenylation sites is boldfaced. The 3'-untranslated region (UTR) of the larger transcript is shown in red to where a poly (A) tail containing 20 'A's is added (not shown) in the smaller transcript.

sites. The same mechanism could well account for the observed different transcript sizes within other species. The canine sequence does not contain the canonical polyadenylation signal (AATAAA); instead a similar sequence motif (ATTAAG) was identified in both appropriate locations (Figure 3). This alternative motif has been demonstrated to serve as a surrogate polyadenylation signal with ~80% efficiency [15]. A non-consensus polyadenylation signal (AATACA) has also been reported to be present in human PDE α cDNA [12].

It is noteworthy that while PDE α cDNA clones from 3 other species contain an ORF capable of coding for a polypeptide of 859 amino acids, the canine PDE α polypeptide is predicted to contain 861 amino acids. With respect to the human and mouse sequences, the two extra amino acids are located as the 849th (Ala) and 850th (Gly) residues (Figure 3). Similar to the comparison made between human and bovine PDE α sequences [12], we noted that the differences in amino acid sequence among the four species (human, mouse, bovine, and canine) are clustered in the 225 N-terminal and 45 C-terminal residues (data not shown). The conserved region includes the domain present in several eukaryotic cyclic nucleotide phosphodiesterases [4]. Alignment of amino acid sequences of canine PDE α and PDE β shows a 72% overall identity, and the domains that are most dissimilar are at the N-terminus (first 50 residues) and C-terminus (last 30 residues).

The data presented here have been used to identify the possible mechanism for the presence of two transcripts for PDE α in different species, and to compare nucleotide/amino acid identity among rod-specific PDE α sequences of these species. Characterization of the wild type canine PDE α cDNA sequence will allow us to detect PDEA mutations in dogs affected with *rcd2* or other inherited retinal degenerations. Because collie dogs affected with *rcd2* have elevated retinal cGMP levels secondary to low PDE activity, we are currently investigating the possibility of a PDE α mutation in this disease.

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The typographical corrections below were made to the article on the date noted. These changes have been incorporated in the article and the details are documented here. 29 March 1999: The text "retinitis pigmentosa (RP)" was changed to "retinitis pigmentosa (OMIM entry 268000)" in the first paragraph of the introduction. The abbreviations "Fig." and "no." were expanded to "Figure" and "number", respectively, throughout the article. The characters enclosing citations to references were changed from parentheses () to brackets []. Several instances of incorrect spacing between a word and punctuation have been corrected.